Journal of Science and Practice of Pharmacy
December 2024; 11 (1): 549-555
Available at http://www.jsppharm.org; https://doi.org/10.47227/jsppharm.v11i1.2
ISSN: 2449-0458 (print); 2449-0466 (electronic)

Original Research Article

Phytochemical screening and CD4 cell count of aqueous extract of *Zingiber officinale* and *Allium sativum* on albino rats

Isonah Unuigbe* and Vincent O Imieje
Departments of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, PMB 1154, Benin City, 300001, Nigeria

* For correspondence: *Email: unuigbe.isonah@uniben.edu. Tel: +2348135645439*

Abstract

Introduction: *Zingiber officinale* and *Allium sativum* are used in traditional medicine to treat cancers, HIV, diabetes, and other diseases. These herbs are claimed to possess immune-modulatory properties. CD4 cells are immune cells that fight infections caused by viruses, bacteria, and other pathogenic organisms.
Purpose: The study aimed to determine the phytochemical constituents of *Zingiber officinale* and *Allium sativum* extracts and to evaluate the immune-boosting effect of the extracts on CD4 cell counts in albino rats.
Methods: The phytochemical screening of the aqueous extracts of powdered ginger rhizome and garlic bulbs was investigated using standard procedures. The CD4 cell count was evaluated after administering oral doses (100 and 200 mg/kg) of each aqueous extract to the rats for 28 days. Changes in the body weights of the experimental animals were monitored weekly using an analytical weighing balance. The animals were sacrificed under chloroform anaesthesia on day 29, and blood samples from each animal were collected from the aorta into EDTA bottles. The samples were analysed for CD4 cell levels using a flow cytometer.
Results: The phytochemical screening revealed alkaloids, carbohydrates, tannins, phenolic compounds, flavonoids, and saponins in *Z. officinale* crude extract. *Allium sativum* extract contains alkaloids, carbohydrates, phenolic compounds, flavonoids, and saponins. The 100 and 200 mg/kg oral doses of the aqueous extracts of ginger and garlic showed increases in CD4 cell levels of 57.2 and 76.0 cells/μL and 60.5 and 62.0 cells/μL, respectively, after treatment with extracts. The treated animals witnessed an increase in body weight compared to the control.
Conclusion: The phytochemicals in *Zingiber officinale* and *Allium sativum* extracts could be responsible for the increased CD4 cell count of albino rats.

Keywords: *Zingiber officinale, Allium sativum,* phytochemical, CD4 count, immune booster

Indexing: Index Copernicus, African Index Medicus

Introduction

Medicinal plants, vaccines, and vitamins have been used as immune modulators, and the immunomodulatory activities of plants have been reported in the literature [1,2]. Plant supplements, such as *Hypericum perforatum* and Astragulus, have been shown to boost the body's immunity [3]. Different defense systems are deployed by the body against invasion by pathogenic microorganisms. CD4 is a glycoprotein found on the surface of immune cells like T-helper cells, macrophages, monocytes, and dendritic cells that function as a co-receptor for the T-cell receptor [4]. CD4 cells are white blood cells, part of the immune system that primarily sends signals to other immune cells to destroy germs or pathogens. CD4 cells represent a unique part of the adaptive immune system that is important in achieving an effective immune response to pathogens. More subsets of CD4 cells with specialised and defined

properties have been identified, like the Tfh and the Th9 [5]. The immune system plays a major role in the body's defences, and a myriad of signalling cascades are involved in the body's immunity. If, for some reason, CD4 cells become depleted, as in the case of HIV infection or following immune suppression before an organ transplant, the body is left exposed to a host of diseases. Proper manipulation of these signalling cascades to target some of the immune cells (neutrophils, macrophages, and lymphocytes) could help in achieving desired therapeutic goals (immune suppression or immune stimulation) [6]. The immuno-stimulatory approach has been used in treating life-threatening conditions such as HIV/AIDS, cancers, and infections, while immune suppression has been harnessed in certain allergies and immune diseases (arthritis, chronic inflammatory diseases, and organ transplants) [7].

Zingiber officinale and *Allium sativum* are medicinal herbs and have been investigated for different conditions, including treating cancers, inflammations, HIV, and other immune deficiency-related conditions [7,8,9]. So far, little is known about how *Zingiber officinale* and *Allium sativum* affect the level of CD4 (T-helper cells) cells in the body. However, some studies have been conducted to determine if the extract from these natural substances affects T-helper cells. One such study tried to evaluate the bioactive compounds in *Zingiber officinale* and how they affect the level of T-helper cells. The potential of this bioactive constituent in increasing the surface molecules of T-cells was investigated using dual tagging FITC (Fluorescein isothiocyanate) and PE (Phosphatidylethanolamine) of monoclonal antibody anti-human with its fluorescence measured with a cytometer [10]. *Zingiber officinale* bioactive constituent increased the ratio of CD3+CD4:CD3+CD8+ T-cells by 30% at 200 μg/mL fraction (oleoresin) concentration. This finding indicates that the bioactive compounds increase cellular and humoral immune responses [11].

The beneficial effect of *Allium sativum* has been emphasised for centuries. It is rich in many bioactive constituents that can affect immunity. Research has shown that *Allium Sativum* possesses some immunomodulatory and anti-inflammatory properties. Research on its effect on immunity showed that it significantly increased the expression of CD8+ lymphocytes in the intraepithelial lymphocytes [12]. Bioactive compounds from *Allium sativum* are effective for relieving symptoms of COVID-19 infection. The virus has been shown to decrease regulatory T cells, cytotoxic cells, helper T cells, and natural killer cells, which may lead to compromised immune responses [13].

Zingiber officinale and *Allium sativum* hold excellent potential as immunomodulators and present a vast field of scientific exploration. There is a lack of studies on the effects of *Zingiber officinale* and *Allium sativum* on CD4 cell count and weight. Hence, this current study aimed to determine the effect of *Zingiber officinale* and *Allium sativum* on weight and CD4 cell count in albino rats

Methods

Materials

All chemicals used in this study were of analytical grade and used as supplied by local vendors. *Zingiber officinale* and *Allium sativum* rhizomes were purchased from a local market in Benin City, Edo State, Nigeria, in May 2023. They were authenticated by Dr. H Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, and a voucher number, UBHD-304, was assigned. The ginger rhizome and garlic bulbs were cleaned, the barks were removed with a knife, and the endoderm was cut into tiny pieces, air dried for 2 weeks under shade, and powdered using a mortar and pestle.

Animals

Twenty-five white Albino rats were purchased from the Department of Pharmacology and Toxicology Animal House, Faculty of Pharmacy, University of Benin, Edo State, Nigeria. The animals were housed in clean plastic cages under a 12-hour light/dark cycle and had access to food and drinking water *ad libitum*. The animals were handled following the international guidelines for using and maintaining experimental animals [14].

Phytochemical screening

Phytochemical screening for secondary plant metabolites (alkaloids, tannins, saponins, carbohydrates, reducing sugars, proteins, flavonoids, and other phenolic compounds) was

performed on powdered samples of the two plants using standard methods [15,16].

Preparation of plant extract

One hundred grams of *Z. officinale* powdered sample was macerated with 500 mL of distilled water for 72 hours. The marc was re-extracted with 300 mL of distilled water and filtered using Whatman No.1 filter paper. Portions of the combined filtrate were concentrated to dryness using an evaporating dish over a hot water bath at 40°C for 6 hours. The recovered concentrate was weighed and stored in a refrigerator at 4°C until needed. A similar process was performed on 85 g of a powdered sample of *Allium sativum* (garlic).

Pharmacological screening

The animals were separated into five plastic cages, with 4 animals in each cage, with free access to food and drinking water. They were grouped and weighed weekly during acclimatisation, and the net weight was noted and recorded. The animals were given calculated doses (100 and 200 mg/kg body weight per oral) of *Z. officinale* and *A. sativum* extracts for 28 days. The dose administered was calculated based on the body weight, as reported by Olaniyan *et al*. [17].

CD4 cell count using a flow cytometer

After the treatment, the animals were sedated under soft chloroform and then euthanised. Using a 5 mL syringe and needle, about 3 – 4 mL of blood was collected from the aorta of each albino rat and transferred into EDTA bottles. The blood samples were sent to the Haematology Laboratory of the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, for CD4 count analysis using a flow cytometer (Partec Cooperation, Germany).

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). A comparison between the treatment and control groups was done using a one-way analysis of variance (**ANOVA**) followed by the Dunnett post hoc test. Analysis and data presentation were done using GraphPad Prism version 8.0.2. Results were considered significant when $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

Phytochemical screening

The extraction yields of the extracts are shown in Table 1, while the results of the phytochemical screening of *Zingiber officinale* and *Allium sativum* showed the presence of alkaloids, carbohydrates, saponins, tannins, phenolic compounds, and flavonoids, while protein was absent (Table 2).

Table 1. Percentage extract yield of *Zingiber officinale* and *Allium sativum* extracts

Test	Percentage yield
Z. officinale	40.37%
A. sativum	39.42%

Table 2. Phytochemical screening results of *Zingiber officinale* and *Allium sativum* extracts

Test	*Z. officinale*	*A. sativum*
Alkaloids	+	+
Carbohydrate	+	+
Saponin	+	+
Tannins	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Proteins	-	-

Where + = present and - = absent

The experimental results showed changes in the body weights of the treated rats, which increased with the duration of treatment. These changes were most significant in the fourth week, especially with the 100 and 200 mg/kg extract of *Z. officinale* and 200 mg/kg of *A. sativum* extract, as shown in Table 3.

A comparison of the weight differences between the treatment groups and the control group weekly is presented in Figures 1-4, while the results of the CD4 cell count via flow cytometry are shown in Table 4.

Table 3: Differences in the experimental animals' weights with time for the duration of treatment

Samples	First week	Second week	Third week	Fourth week
Control (Distilled water)	128.3 ± 16.0	143.7 ± 19.07	156.0 ± 20.38	173.4 ± 3.23**
Z. officinale (100 mg/kg)	143.5 ± 12.70	160.1 ± 14.81	184.7 ± 17.27**	204.3 ± 19.48***
Z. officinale (200 mg/kg)	153.1 ± 11.70	168.0 ± 12.13	173.1 ± 13.01	204.5 ± 25.40***
A. sativum (100 mg/kg)	155.6 ± 16.38	149.3 ± 12.16	182.3 ± 18.91	202.7 ± 21.39**
A. sativum (200 mg/kg)	144.3 ± 14.74	152.4 ± 12.88	172.4 ± 13.35*	191.8 ± 17.37***

Values are expressed in mean ± SD. All weeks were compared against Week 1, and the result was considered statistically significant when $p < 0.05$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

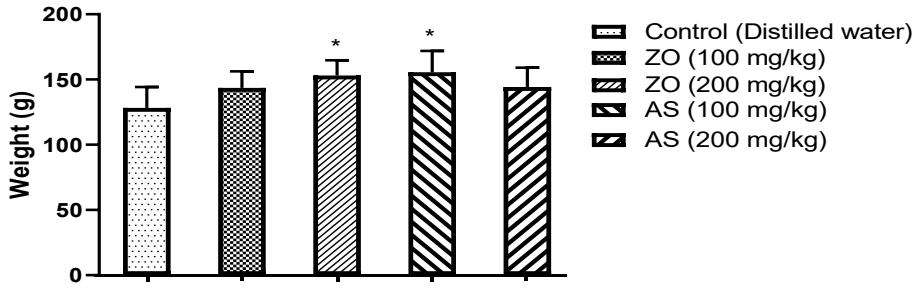

Figure 1: Week 1 weight variations among the treatment groups given 100 and 200 mg/kg bw of the extracts and control (distilled water). The result was considered statistically significant when **p*< 0.05.

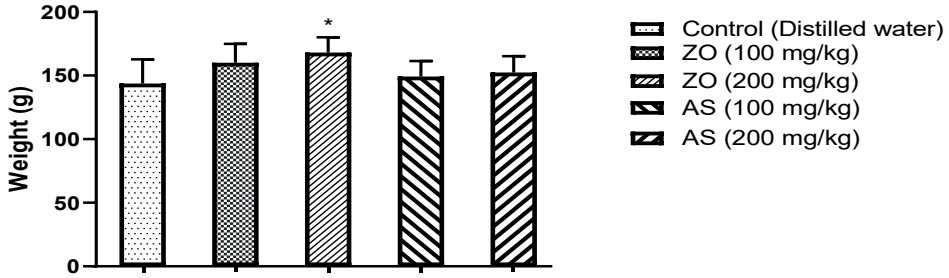

Figure 2: Week 2 weight variations among the treatment groups given 100 and 200 mg/kg bw of the extract and control (distilled water). The result was considered statistically significant when **p*< 0.05.

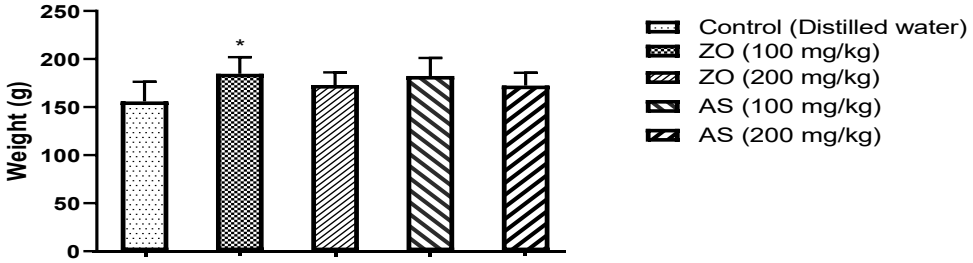

Figure 3: Week 3 weight variations among the treatment groups given 100 and 200 mg/kg bw of the extract and control (distilled water). The result was considered statistically significant when **p*< 0.05.

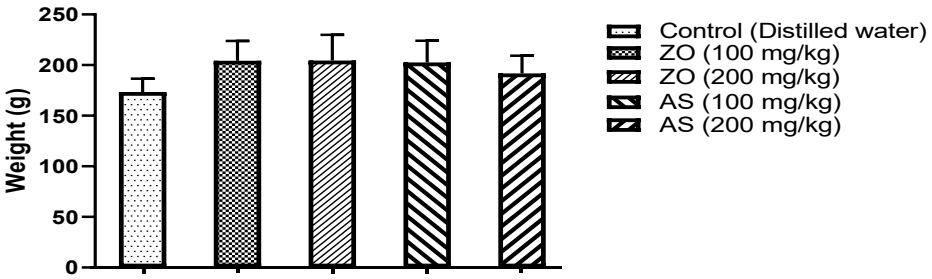

Figure 4: Week 4 weight variations among the treatment groups given 100 and 200 mg/kg bw of the extract and control (distilled water). The result was considered statistically significant when **p*< 0.05.

Table 4: Results of CD4 cell counts of the experimental rats in each group.

Groups	Dose	Mean CD4+ Count (cell/mm^3)
Control (Distilled water)	5 mL	44.3±12.66
Z. officinale (100 mg/kg)	100 mg/kg	57.2±33.16
Z. officinale (200 mg/kg)	200 mg/kg	76.0±42.09
A. sativum (100 mg/kg)	100 mg/kg	60.5±11.12
A. sativum (200 mg/kg)	200 mg/kg	62.0±9.11

Values are expressed in mean ± SD. All treatment groups were compared with the control group, and the result was considered statistically significant when **p* < 0.05.

NOTE: The results of the statistical analysis revealed no significant difference between groups in terms of CD4 counts following treatment.

Discussion

Plants have been identified for the treatment of cancer, diabetes, infections, HIV/AIDS, and inflammation [18]. Generally, the method used to extract plant materials plays a crucial role in the bioactivity screening of the extracts because it determines the concentration and polarity of the secondary plant metabolites present. The percentage yields of 40.37% and 39.42% obtained from the aqueous extraction of the powdered rhizomes of *Zingiber officinale* (100 g) and *Allium sativum* (85 g) were high when compared to values obtained in other methods with other medicinal plants [19]. The maceration method used for the extraction process uses simple materials and is very suitable for thermolabile plant materials since it does not involve high temperatures [20].

The pharmacological activities of *Zingiber officinale* and *Allium sativum* are due to various secondary plant metabolites, alkaloids, saponins, tannins, phenolic compounds, and flavonoids. Alkaloids and polyphenols are known for their biological activities, such as antioxidant, muscle relaxant, antimicrobial, anticancer, and anti-diabetic activities. These phytochemicals in the plants studied (*Zingiber officinale* and *Allium sativum*) may have been responsible for the immune-boosting effects of these plants and the increase in the CD4 cell count.

On the other hand, tannins have been reported to form complexes with proteins and inactivate microbial adhesion enzymes, cell envelope, and transport proteins. They also form complexes with polysaccharides. Studies have shown tannins to inhibit viral reverse transcriptase [21] linked to secondary metabolites in the plant material.

Many plants have been reported to possess immune activation properties [22,23]. CD4 cells (CD4+ T cells) are white blood cells that ward off infections in the body. CD4 cell levels (count) are an indicator of immune function in HIV patients and one of the critical determinants of the need for opportunistic infection prophylaxis. In established HIV infection, there is an increased susceptibility of the patient to opportunistic infections and tumours due to the loss of memory CD4+ T cell reactivity against recall antigens, an early event in HIV disease progression [24]. Plant extracts have shown *in vitro* immunomodulatory potential in inhibiting CD4+ T-cell activation, and extracts of *Azadirachta indica* have demonstrated their ability as a source of natural products for targeting persistent immune activation and inflammation during antiretroviral therapy [25]. In a review by Sultan *et al.* (2014), *Zingiber officinale* and *Allium sativum* were listed among medicinal plants with immune-boosting potentials due to the presence of functional ingredients (alkaloids, polyphenolics, flavonoids, terpenoids, etc.) [22].

Results of this current study showed a linear association between the mean weight of the rats and their CD4+ count, with the rats of high weight having a high value of CD4 count, suggesting the reason for the high mean CD4 count recorded in the treatment group (Table 4). All the extracts caused an increase in the body weight of the experimental animals, which was pronounced in week four of treatment with $p < 0.001$ (Table 2). There were significant ($p < 0.05$) differences in the body weight of the treatment groups compared with the control, especially with *Zingiber officinale* at 100 and 200 mg/kg body weight (Figures 3 & 4).

Conclusion

Zingiber officinale and *Allium sativum* powder contain phytoconstituents that affect the CD4 count of albino rats and can be beneficial as supplements to boost the immunity of healthy individuals. The plant extracts influenced weight gain in Albino rats. This effect may be vital in persons living with HIV and other immunodeficiency diseases. However, there is a need for further studies to evaluate the immune-boosting potential of the extracts in induced immunocompromised animals.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. UI conceived, designed, and supervised the study. UI and VIO collected and analysed the data and prepared the manuscript. All the authors read and approved the final draft submitted.

Ethical Approval

Ethical approval (EC/FP/018/33) was obtained from the Ethical Committee, Faculty of Pharmacy, University of Benin, before the commencement of this study. The animals were handled according to established protocols of the ethical committee of the Faculty of Pharmacy, University of Benin, on the care and use of experimental animals.

Acknowledgments

The authors thank Mr. Ibe and Mr. Kingsley from the Department of Pharmacology and Pharmacognosy, respectively, Faculty of Pharmacy, University of Benin.

References

1. Zebeaman M, Tadesse MG, Bachheti RK, Bachheti A, Gebeyhu R, Chaubey KK. Plants and plant-derived molecules as natural immunomodulators. Biomed Res Int. 2023;2023:7711297.
2. Ilyas U, Katare DP, Aeri V, Naseef PP. A review on hepatoprotective and immunomodulatory herbal plants. Phcog Rev. 2016;10(19):66.
3. Abbas A.K., Lichtman A.H., Pillai S. Functions and Disorders of the Immune System. Basic Immunology. 6th Edition: Amsterdam: Elsevier, 2016, 25: 3-7
4. Young NA, Al-Saleem T. Lymph nodes: Cytomorphology and flow cytometry. In Comprehensive Cytopathology. 2008; 671-711. WB Saunders.
5. Owens JA, Punt J, Stranford SA, Jones PP (2013). Kuby Immunology (7th ed.). New York: W.H. Freeman. pp. 100–101
6. Chauhan RS, Singh GK. Immunomodulation: An overview. J Immunol Immunopathol. 2001;3(2):1-5.
7. Bascones-Martinez A, Mattila R, Gomez-Font R, Meurman JH. Immunomodulatory drugs: Oral and systemic adverse effects. Med Oral Patol Oral Cir Bucal. 2014;19 (1):e24-31.
8. Shahrajabian MH, Wenli SU, Cheng Q. Pharmacological uses and health benefits of ginger (*Zingiber officinale*) in traditional Asian and ancient Chinese medicine, and modern practice. Not Sci Biol, 2019, 11(3):309-319.
9. El-Saber Batiha G, Magdy Beshbishy A, G. Wasef L, Elewa YH, A. Al-Sagan A, Abd El-Hack ME, Taha AE, M. Abd-Elhakim Y, Prasad Devkota H. Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. Nutrients. 2020;12(3):872.
10. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, Li HB. Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). Foods. 2019;8(6):185.
11. Tejasari D. Evaluation of ginger (*Zingiber officinale* Roscoe) bioactive compounds in increasing the ratio of T-cell surface molecules of CD3+ CD4+: CD3+ CD8+ *in-vitro*. Malays. J. Nutr. 2007;13(2):161-70.
12. Arreola R, Quintero-Fabián S, López-Roa RI, Flores-Gutiérrez EO, Reyes-Grajeda JP, Carrera-Quintanar L, Ortuño-Sahagún D. Immunomodulation and anti-inflammatory effects of garlic compounds. J Immunol Res. 2015;2015:401630.
13. Khubber, S., Hashemifesharaki, R., Mohammadi, M. *et al.* Garlic (*Allium sativum* L.): a potential unique therapeutic food rich in organosulfur and flavonoid compounds to fight with COVID-19. Nutr J. 2020; 19, 124.
14. Organization for Economic Cooperation and Development (OECD): (2001): Environment, Health and Safety Publications Series on Testing and Assessment, No 24: Guidance Document on Acute Oral Toxicity Testing.
15. Sofowora A. Screening Plants for Bioactive Agents. In Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan:1982. pp 128-161
16. Evans WC. Trease and Evans Pharmacognosy, 15th ed., Churchill Livingstone Harcourt Publishers Limited, London: 2002. pp 204-393
17. Micheal AO, Folaranmi OM, Ajoke OD, Olatunji AG, Oluwole A, Adewumi A. Effects of ginger (*Zingiber officinale* Roscoe) and garlic (*Allium sativum* L.) on rats infected with *Klebsiella pneumonea*. Int J Altern Med. 2009;7(1):346-439.
18. Jongrungraungchok S, Madaka F, Wunnakup T, Sudsai T, Pongphaew C, Songsak T, Pradubyat N. *In vitro* antioxidant, anti-inflammatory, and anticancer activities of the mixture of Thai medicinal plants. BMC Complement Med Ther. 2023;23(1):43.
19. Spyrou A, Batista MG, Corazza ML, Papadaki M, Antonopoulou M. Extraction of high value products from *Zingiber officinale* Roscoe

(Ginger) and utilization of residual biomass. Molecules. 2024;29(4):871.

20. Tavs AA, Ukamaka OL. Antimicrobial and anti-inflammatory activities of *Annona muricata* L. (Annonaceae) formulated as an herbal toothpaste. J Sci Pract Pharm. 2023;10(1):533-42.
21. Huang Q, Liu X, Zhao G, Hu T, Wang Y. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Anim Nutr. 2018;4(2):137-150.
22. Sultan MT, Butt MS, Qayyum MM, Suleria HA. Immunity: plants as effective mediators. Crit Rev Food Sci Nutr. 2014;54(10):1298-308.
23. Mallikarjuna K, Sahitya Chetan P, Sathyavelu Reddy K, Rajendra W. Ethanol toxicity: rehabilitation of hepatic antioxidant defense system with dietary ginger. Fitoterapia. 2008;79(3):174-178
24. Sanna C, Marengo A, Acquadro S, Caredda A, Lai R, Corona A, Tramontano E, Rubiolo P, Esposito F. *In vitro* anti-HIV-1 reverse transcriptase and integrase properties of Punica granatum L. leaves, bark, and peel extracts and their main compounds. Plants. 2021;10(10):2124.
25. Olwenyi OA, Asingura B, Naluyima P, Anywar GU, Nalunga J, Nakabuye M, Semwogerere M, Bagaya B, Cham F, Tindikahwa A, Kiweewa F. In-vitro Immunomodulatory activity of Azadirachta indica A. Juss. Ethanol: water mixture against HIV-associated chronic CD4+ T-cell activation/exhaustion. BMC complementary medicine and therapies. 2021; 21:114.